Bayer HealthCare
Bayer Schering Pharma

Department: **GDD-GED Toxicology** **GLP Report**

Report No.: AT06113

Test Item: **PES Vorstufe 2342**

Title: **Acute Skin Irritation/Corrosion on Rabbits**

Study No.: T 0081344

Author(s): **C. Gmelin**

Study Completion Date: November 12, 2010

Performing Laboratory
Bayer Schering Pharma AG
GDD-GED Toxicology
42096 Wuppertal
Germany

Sponsor
Bayer MaterialScience AG

51368 Leverkusen
Germany

GLP Compliance Statement

The study was conducted in compliance with the principles of Good Laboratory Practice described in the following issues:

- OECD Principles of Good Laboratory Practice (as revised in 1997) [ENV/MC/CHEM(98)17]

- Bulletin of the revised form of the chemicals act of July 2, 2008, Federal Law Gazette Volume 2008 Part I No. 28, section 6, §19, issued at Bonn July 11, 2008)

C. Gmelin
(Study Director)

Oct. 15, 2010
Date

Quality Assurance Statement

Study No.: T0081344

Test Item: PES Vorstufe 2342

On the dates given below inspections were conducted by the Quality Assurance to ensure that no deviations exist that are likely to affect the integrity of this study.
The Quality Assurance Unit monitors the conduct of each study by study-based inspections or by process-based inspections of a similar type of study if the short-term nature of a study precludes inspection while it is in progress. Routine procedures and the equipment used in the relevant laboratory areas are inspected regularly and reports are made in accordance with current SOPs.

*(study plan amendments, if any, were duly audited and reported to the Study Director and Management)

Date of Audits / Inspections	**Phases Audited / Inspected**		**Date of Report to Study Director and Management**
Aug-23-2010	Study Plan *		Aug-23-2010
Aug-24-2010	process based	Administration / Dosing, Weighing, Raw Data / Documentation	Aug-24-2010
Oct-26-2010	Main Report	1. Draft	Oct-26-2010
Oct-26-2010	Main Report	Final Draft	Oct-26-2010

The results of this study including the methods used have been checked on the basis of the current SOPs.
They have been correctly reported and the report reflects the raw data.
In case of a multi-site study audits at the test sites are presented in the QA Statement of the Principal Investigator's report (see appendix).

Quality Assurance Unit
Global R&D Quality, GLP-Mgmt.

Date: Oct-26-2010

Signature: Ursula Turek
Ursula Turek

Signatures

Study Director: C. Gmelin (C. Gmelin) Nov. 12, 2010 Date

Senior Expert: (Prof. Dr. H.-W. Vohr) Nov. 12, 2010 Date

Table of Contents

1. Summary

This study was performed to assess potential irritant / corrosive effects of PES Vorstufe 2342 (purity: 100 %) on the skin of rabbits.

The results are summarized in the table below.

Table 1-1 Summary of Irritant Effects (Score)

Animal		24 h	48 h	72 h	Mean scores	Response	Reversible (days)
1	Erythema (redness) and eschar formation	1	1	1	1.0	-	7
	Oedema formation	0	0	0	0.0	-	na
2	Erythema (redness) and eschar formation	1	1	0	0.7	-	3
	Oedema formation	0	0	0	0.0	-	na
3	Erythema (redness) and eschar formation	1	1	1	1.0	-	7
	Oedema formation	0	0	0	0.0	-	na

no positive response : mean scores < 2 = -
positive response : mean scores ≥ 2 = +
na : not applicable

Conclusion:

According to classification criteria PES Vorstufe 2342 is not an irritant to the skin.

There were no systemic intolerance reactions.

2. Introduction and Guidelines

The study objective was to determine the irritant / corrosive effects on skin of albino rabbits. Information derived from this test serves to indicate the possible existence of hazards likely to arise from short-term exposure of skin to the test substance, and - with respect to a proper handling and use - serves to permit classification (labelling) of a product.

The method used complied with the OECD - Guideline for Testing of Chemicals No. 404 -" Acute Dermal Irritation/Corrosion"; adopted: 24th April 2002 and the EEC Directive No. 440/2008 Part B - Method B.4.

For reasons of animal welfare a sequential testing strategy was followed in accordance with the current version of the EEC Directive No. 440/2008 and the OECD Guideline No. 404, irrespective of the requirements of other guidelines for testing for dermal irritation/corrosion in rabbits.

This testing strategy comprised a stepwise approach including the evaluation of existing human and/or animal data showing effects on the skin or mucous membranes, the performance of a SAR evaluation for skin corrosion/irritation, measurement of pH value, the evaluation of data on systemic toxicity via the dermal route and the performance of a validated in vitro test for skin corrosion and an in vitro test for skin irritation (Human 3D Epidermal Skin Model) before in vivo testing for skin irritation/corrosion in rabbits.

The in vitro test for skin corrosion is archived under T 4081285. The test compound is not corrosive to the skin. The in vitro test for skin irritation is archived under T 3081284. The test compound is not irritating to skin.

3. General Information

The study was sponsored by Bayer MaterialScience AG, 51368 Leverkusen, Germany.

The study was performed at Bayer Schering Pharma AG, GDD-GED Toxicology, 42096 Wuppertal, Germany.

3.1 Key Study Data

Study No.:	T 0081344
Study initiation date:	2010-08-19
Experimental starting date:	2010-08-24
Experimental completion date:	2010-08-31
Study completion date:	see signature page

3.2 Archiving

The study protocol, raw data and final report are retained in the archives specified by the test facility Toxicology of the Bayer Schering Pharma AG in Wuppertal. A retention sample of the test item was stored in the archive of the test facility.

3.3 Responsibilities

Study Director:	C. Gmelin
Senior Expert:	Prof. Dr. H.-W. Vohr
Test Facility Management:	Dr. T. Steger-Hartmann
Head of Test Facility:	Dr. F.-W. Jekat
Archiving:	R. Zils
Head of Quality Assurance Unit:	Dr. A. Paeßens

4. Material and Methods

4.1 Test Item

Test item:	PES Vorstufe 2342
Synonym(s):	Ester Rizinus + Sojaoel-Umesterung
EC No.:	919-697-6
Chemical name:	Castor Oil, reaction product with Soybean Oil
Batch no.:	LB06603520
Appearance:	colorless liquid
Content of test item*:	100 % (dosing is based on the test item)
Storage*:	refrigerator, amber bottle
Expiry date:	2010-10-22

*due to product information given by the sponsor

The documentation of the chemical composition of the test item is in the responsibility of the sponsor. Confirmation of the identity of the test item was performed.

4.2 Experimental Animals

The study was performed in female albino rabbits (strain Crl:KBL(NZW)BR, Charles River, 88353 Kißlegg, Germany) recommended as the preferred species for this type of studies.

Healthy adult albino rabbits free of clinical signs were used. The health of the animals was routinely examined for the main specific pathogens by the breeder. Before the study the rabbits had been vaccinated against RHD (rabbit haemorrhagic disease). No treatment with antibiotics was performed prior to receipt of the animals, or during the acclimatization phase or study period. In some cases the animals had already been used in earlier studies. This has no influence to the results of this study. Females were nulliparous and not pregnant. The acclimatization time was at least 5 days.

Body weights at start of study:
3.5 kg – 3.6 kg

Age: young adult animals

The animals were identified by labels on the cages stating at least study number, test compound, animal number and by tattooed number assigned by the breeder.

4.2.1 Husbandry and Nutrition

The animals were housed individually in cage units Metall/Noryl by EBECO. Excrement trays below the cages contained low dust wood granulate bedding (J. Rettenmaier & Söhne, 73494 Rosenberg, Germany). The wood granulate was changed at least twice weekly. The animals were regularly transferred to clean cages.

The animal room had a standardized climate:

Room temperature: 20 ± 3°C

Air humidity: 50 ± 25 %

Light/ Dark cycle: 12 hours rhythm.

The humidity and air temperature were continuously recorded. Occasionally deviations from the standards occurred, e.g. during cleaning of the animal room or effects of the weather. They did not have any apparent influence on the outcome of the study. The animal room was provided with sound from a radio program.

The animals received the standard diet "Ssniff K-Z" 4mm (manufacturer: Ssniff Spezialdiäten GmbH, 59494 Soest, Germany), approximately 100 g per animal per day and tap water ad libitum from polycarbonate bottles. To satisfy the needs of roughage, hay was offered additionally (hay, irradiated, delivered by Harlan Nederland, 5961 NM Horst, Netherland respectively hay pellets, manufacturer: ssniff Spezialdiäten GmbH, 59494 Soest, Germany).

The nutritive composition and the contaminant content of the standard diet were checked and analyzed routinely in random samples. No unwanted ingredients were detected. The tap water was of drinking water quality (according to the Drinking Water Decree in the current version) and analyzed routinely. The wood granulate was randomly checked for contaminants at regular intervals.

The results of these analyses have been stored at Bayer HealthCare AG, 42096 Wuppertal, Germany. The available data yielded no evidence of any adverse effects on the aim of the study.

The animal room was cleaned at least once a week and disinfected at least once a month. It was ensured that the diet was not contaminated, and that there was no contact of the cleaning or disinfecting solution with the test animals.

4.2.2 Number of Animals

Three animals were used for the study.

4.3 Exposure Procedure

On the day before the test, the fur was shorn on the right and left side from the dorso-lateral area of the trunk of each of the rabbits. Care was taken to avoid abrading the skin. Only animals with healthy and intact skin were used.

0.5 ml of the pure liquid test substance was applied to the skin of the animal under a gauze patch. The treated skin area was approximately 2.5 cm by 2.5 cm in size. The patch was placed on the dorso-lateral areas of the trunk of each animal and was held in place with non-irritant tape for the duration of the exposure period. Thus, access by the animal to the patch and resultant ingestion of the test substance was prevented.

After the exposure period the dressing and patch were removed. The exposed skin area was carefully washed with water without altering the existing response, or the integrity of the epidermis. The surrounding untreated skin served as control.

Due to a possible irritant potential of the test substance, in the first step only one animal was used and three test patches were applied successively to this animal, as described above. The first patch was removed after three minutes. As no serious skin reactions were observed, the second patch was applied and removed after one hour. At this stage the observations indicated that with respect to animal welfare the exposure can be allowed to extend to four hours, therefore the third patch was applied and removed after four hours and the responses were graded one hour later. The test was completed using two additional animals, exposed for four hours.

4.4 Observations and Scoring

Based on most recent guidelines the dermal irritation was scored approximately at 1, 24, 48 and 72 hours after patch removal. If no irritation indices were observed after 72 h, the study was finished. If dermal irritation was observed, animals were monitored usually on day 7 and 14 after patch removal until the changes had completely subsided, however for not more than 14 days after application.

The degree of erythema/eschar formation and oedema formation was recorded as specified by DRAIZE (see Annex page 20) and any serious lesions or toxic effects other than dermal irritation were also recorded and fully described.

As general criteria the body weight of each animal was recorded at the beginning of the study. If clinical findings other than dermal irritations occur they were recorded daily.

4.5 Evaluation and Interpretation of Results

The interpretation of the results is based on the EEC Directive No. 440/2008 Part B - Method B.4. (see Annex page 21).

5. Results

The individual findings of the treated skin areas at the various observation times are summarized in the tables below. The control areas did not show any abnormal findings and are not listed in the table.

Table 5-1 Irritant Effects on the Skin - First Animal (Exposure 3 minutes / 1 hour)

Animal 1 Observation (immediately after patch removal)	Exposure 3 minutes	Exposure 1 h
Erythema (redness) and eschar formation	0	0
Oedema formation	0	0

Animal 1; exposure 3 min. and 1h, immediately after patch removal:

test compound could not be removed completely from the skin, residues of the test item adhered to the treated skin area

Table 5-2 Irritant Effects on the Skin (Exposure: 4 hours)

Animal 1, Body Weight 3.6 kg Observation (after patch removal)	1 h	24 h	48 h	72 h	day 7	day 14
Erythema (redness) and eschar formation	0	1	1	1	0	-
Oedema formation	0	0	0	0	0	-
Animal 2, Body Weight 3.5 kg Observation (after patch removal)	1 h	24 h	48 h	72 h	day 7	day 14
Erythema (redness) and eschar formation	0	1	1	0	-	-
Oedema formation	0	0	0	0	-	-
Animal 3, Body Weight 3.6 kg Observation (after patch removal)	1 h	24 h	48 h	72 h	day 7	day 14
Erythema (redness) and eschar formation	0	1	1	1	0	-
Oedema formation	0	0	0	0	0	-

- : no further examination

Animal 1, 2 and 3; 1h after patch removal:

test compound could not be removed completely from the skin, residues of the test item adhered to the treated skin area

Table 5-3 Summary of Irritant Effects (Score)

Animal		24 h	48 h	72 h	Mean scores	Response	Reversible (days)
1	Erythema (redness) and eschar formation	1	1	1	1.0	-	7
	Oedema formation	0	0	0	0.0	-	na
2	Erythema (redness) and eschar formation	1	1	0	0.7	-	3
	Oedema formation	0	0	0	0.0	-	na
3	Erythema (redness) and eschar formation	1	1	1	1.0	-	7
	Oedema formation	0	0	0	0.0	-	na

no positive response : mean scores < 2 = -
positive response : mean scores ≥ 2 = +
na : not applicable

6. Conclusion

The irritant / corrosive potential of PES Vorstufe 2342 was studied on the skin of rabbits. The method used complied with the OECD - Guideline for Testing of Chemicals No. 404 -" Acute Dermal Irritation/Corrosion"; adopted: 24th April 2002 and the EEC Directive No. 440/2008 Part B - Method B.4.

According to classification criteria PES Vorstufe 2342 is not an irritant to the skin.

There were no systemic intolerance reactions.

Ministerium für Arbeit, Gesundheit und Soziales
Des Landes Nordrhein-Westfalen

Fürstenwall 25, 40219 Düsseldorf — Aktenzeichen II A 5 – 31.11.46.06

Gute Laborpraxis/Good Laboratory Practice
GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Bayer HealthCare AG
BSP-GDD-GED
Toxikologie
Aprather Weg 18 a
42096 Wuppertal

Prüfungen nach Kategorien (gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)	Areas of Expertise (according ChemVwV GLP Nr. 5.3/OECD guidance)
Kategorie 1 Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	**category 1** physical-chemical testing
Kategorie 2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	**category 2** toxicity studies
Kategorie 3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	**category 3** mutagenicity studies
Kategorie 9 Biochemische Toxikologie; Kurzzeitkanzerogenese; Immuntoxikologie; Sicherheitspharmakologie	**category 9** biochemical toxikology; short time cancerogenicity; immunotoxicity; safety pharmacology
Datum der Inspektion 01.Sept.2008 bis 05.Sept.2008	**Date of Inspection** September 1st 2008 until September 5th 2008

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

The above mentioned test facility/ test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Düsseldorf, den 09.02.2009
Im Auftrag

(Dr. Deden)

Dienstsiegel/official-seal

Grading of Skin Reactions	
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beefy redness) to eschar formation preventing grading of erythema	4
Maximum possible: 4	
Oedema Formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum possible: 4	
Corrosive	
Irreversible damage of the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to four hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars.	C

Interpretation of Results
Causes severe burns A test material that causes severe burns to skin is a test material that, if when applied to healthy intact animal skin, results in - full thickness destruction of skin tissue as a result of up to three minutes exposure **Causes burns** A test material that causes burns to skin is a test material that, if when applied to healthy intact animal skin, results in - full thickness destruction of skin tissue as a result of up to four hours exposure.
Irritating to skin A test material with irritating potential to skin is a test material that produces at least in 2 of 3 tested animals a positive response of: - erythema/eschar* ≥ 2 and/or - oedema* ≥ 2 and/or - inflammation persisting until the end of the observation time.
*: calculated as the mean scores at the reading times 24, 48 and 72 hours.